Non-Markovian Gating of Ca^{2+} -Activated K^{+} Channels in Cultured Kidney Cells *Vero*. Rescaled Range Analysis

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Abstract. Using the patch-voltage clamp technique and the rescaled range method, activity of single large conductance Ca^{2+} -activated K^{+} channels (K_{Ca} channels) was studied. For the sequences of alternating open and shut time intervals, the dependence R/S vs. N_{τ} in the double logarithmic coordinates presented a curve with two slopes, $H_1 = 0.60 \pm 0.04$, and $H_2 = 0.88 \pm 0.21$, where H_1 and H_2 characterized the Hurst exponents for short and long time ranges, respectively. Similar results were obtained for reduced data sets consisting of only open or only shut intervals. Randomization of the experimental data resulted in a single slope, H , of 0.52 ± 0.02 . Simulations were performed with eight-state Markovian model without memory. The calculated Hurst exponent presented in average 0.54 ± 0.02 . The results suggest that the activity of single Ca^{2+} -activated K^{+} channel exhibits two regimes, with slight positive correlation at short time ranges ($H_1 = 0.6$), and strong positive correlation at long time ranges ($H_2 = 0.88$); therefore the channel gating as a whole is not a steady-state Markovian process.

Key words: *Vero* cells, Ca^{2+} -activated K^{+} channel, Patch-voltage clamp, Rescaled range analysis.

Introduction

Most studies of channel gating kinetics have assumed random fluctuations of single channel between a finite number of discrete states, with the time-independent rates of transitions among the states (for references see [1–3]). Discrete states with transitions rates independent of previous channel activity define a discrete Markov gating process. Some support for Markov gating process has been obtained (for references see [1–3]).

McManus and co-authors [1, 4, 5] have found inverse relationships of the durations of adjacent open and shut intervals (K_{Ca} channels in cultured rate skeletal muscle and other channel types were tested). However, the rate constants for channel transitions between the states were found to be independent of adjacent interval duration, and hence, previous channel activity. Observed correlations of adjacent

single channel dwell-times together with Markov gating indicates that there are two or more independent transition pathways connecting open and shut states.

On the other hand, some experimental data could be interpreted to indicate the channel kinetic is a process with memory, that is, the rate constants of the channel transitions between the states depend on previous channel activity. The process with memory may have different (e.g., fractal) rather than Markovian statistics. Some indications of fractal properties of single channel activity have been recently obtained [6–8], and fractal concepts were used to analyze the sequences of open and shut times [9, 10].

A few techniques have been developed for analysing fractal time series, rescaled range analysis being one of them [11]. The method is comparatively simple and rather faithful [12]. The rescaled range R/S is the range of cumulative deviations from the mean, divided by the standard deviation S , where estimates of R/S are obtained for subsets of durations τ for many different τ 's. When the data exhibit self-similarity and fractal correlation, then:

$$\frac{R}{S}(\tau) = (a\tau)^H \quad (1)$$

where a is a constant (frequently, $\pi/2$) and H – is the Hurst exponent. H gives a measure of the smoothness of a fractal process, with $0 \leq H \leq 1$. It has been shown that for mutually independent random variables H exactly equals 0.5 [13]. When $H \neq 0.5$, a process has memory. If $H > 0.5$, a process is called persistent because an increase in the present is more likely to be followed by a later increase. If $H < 0.5$, the function is antipersistent, because an increase in the present is more likely to be followed by a later decrease.

Hurst [11] himself have found that for many natural phenomena H is, in average, about 0.73, that is, the phenomena exhibited correlated rather than random time series. Many biological process were succesfully examined for their fractal nature by different statistical measures, but the Hurst method was used only in a few cases. Recently Teich et al. [14] have found that the neural spike train in the visual system of the cat has properties of a process whose mean is modulated by fractal binomial noise. The findings of Churilla et al. [15] indicate that the membrane potential fluctuations of human T-lymphocytes have fractal characteristics of fractional Brownian motion. The process has two regimes: at short lags $H = 0.76$ (persistent correlation), and at long lags $H = 0.26$ (antipersistent correlation). Activity of unitary ion channels has been analysed by the R/S method, to our knowledge, in the sole case, when Nogueira et al. [8] have shown that for the Ca^{2+} -activated K^+ channels of Leyding cells, the Hurst exponent was equal to 0.75, that is the successive openings and closings were assumed to be a process with memory.

We used the Hurst method to examine the data obtained on the single large conductance Ca^{2+} -activated K^+ channels in cultured kidney cells. The analysis was aimed at revealing time correlation, if it exist, in the apparently random fluctuations of the channel gating, and investigation of the Hurst exponent behaviour for

different time series. The findings were compared with those obtained on simulated data for the model eight-state Markovian channel.

1. Materials and Methods

Experiments were performed on cultured kidney cells *Vero*. Experimental procedures, data record, and analysis were described earlier [16]. Some relevant experimental details are as follows. The temperature of the experimental solutions was maintained at 22 °C. Single channel currents from ‘inside-out’ patches were recorded at a cut-off frequency of 1 to 3 kHz (‘dead’-time equals 70–200 μ s). The samples with basically normal mode of channel kinetics [17] and stable current baseline were selected for analysis at a sampling period of 0.1 ms. Durations of open (τ_o) and closed (τ_c) intervals were determined at a 50% level of the channel current amplitude, using the software created by F. Iljasov.

Rescaled range analysis (R/S -analysis, Hurst analysis) was used to study temporal records of the K_{Ca} channel activity representing sequences of alternating open and shut intervals. Besides, reduced sequences of either only open or only closed events were used. The channel activity was described by a time-dependent function $F(\tau)$ with τ assuming only integer values (1, 2, $\dots$, τ , $\dots$, T). Standard R/S analysis with linear regression in double-logarithmic coordinates was used [12]. The Hurst exponents were calculated with the following procedures). 1) An initial (general) data set of T events (adjacent open and shut time intervals) was divided into samples (subsets) of N_τ events ranging in size from 2^3 up to $2^n = T$ events. T was varied from several hundreds to several tens of thousands depending of an experimental task. 2) The first R/S mean value was calculated for $T/2^3$ arrays each of 2^3 events, the second mean value of R/S was calculated using $T/2^4$ arrays each of 2^4 events, and so on until a single sample of $T = 2^n$ data points was reached. 3) The mean R/S values were plotted against N_τ in a double-logarithmic plot. 4) The values of the Hurst exponent was then calculated as a slope of the dependence R/S vs. N_τ using standard linear regression. A straight line on this plot indicated that there may be self-similar correlation at all scales. The method gives estimated H values which at correct analysis are higher than the true H values by a few percent [12].

For comparison, the R/S -analysis was applied to the randomized data sets and to the simulated data from a Markovian model of a channel.

Random shuffling of a data set consisting of 2^n time intervals, τ , was made in the following manner. The data set was brought into the compliance with an array of 2^n random numbers, r , generated by a computer. Then, the numbers in all pairs r_i and r_{i+1} were compared, and if r_{i+1} were larger than r_i the numbers would be interchanged. Corresponding interchanges were made in the data sample. The procedure was repeated for each reduced series of τ and r consisting of $2^n - 1$, $2^n - 2$, $\dots$, and 2 events. As a result, a randomized data set was obtained.

The eight-state Markovian model was developed by McManus and Magleby [18] for the large-conductance Ca^{2+} -activated K^+ channel in rat skeletal muscle. This channel resembled the K_{Ca} channel in the *Vero* cells [16]. The kinetic model was given by a scheme with five closed and three open states:

Here C and O represent closed and open states, respectively. The data were simulated using algorithm described in [19]. The simulated single-channel current records were generated with various levels of time resolution, ranging from infinite time (no filtering) to highly limited time resolution (filtering at 100 Hz).

2. Results and Discussion

Earlier two main kinetically distinct, fast and slow, types of large-conductance Ca^{2+} -activated K^+ channels (K_{Ca} channels), have been identified and characterized in the *Vero* cells [16]. No noticeable differences were found when the *R/S*-analysis was applied either to the fast or slow K_{Ca} channels. A characteristic property of the K_{Ca} channels is that their activity enhanced at shifting of the membrane potential (V_m) to positive values and increasing internal (bath) Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). At several μM of $[\text{Ca}^{2+}]_i$ and V_m in the vicinity of zero, the channel activity presented short (about 1 ms) current impulses alternated with long shut intervals. At higher values of $[\text{Ca}^{2+}]_i$ and V_m , the channel exhibited bursting activity (Figure 1a).

Single channel activity and R/S-analysis. Figure 1 shows activity of a single fast K_{Ca} channel which appears as a sequence of single current pulses and bursts. The *R/S*-analysis was applied to three groups of the data sets: 1) the original sequences of alternating shut and open intervals, 2) the sequences of only open intervals, and 3) the sequences of only shut intervals. Figure 1b shows a typical *R/S* vs. N_τ dependence for an original data set of adjacent open and closed times (about 10^5 events). In the double-logarithmic coordinates, the experimental points can be fitted by two straight lines with different slopes rather than by an expected single straight line. The linear regression gives a slope (the Hurst exponent) of the initial part of the curve $H_1 = 0.58 \pm 0.01$, and a slope of the second curve part $H_2 = 0.99 \pm 0.04$. The two zones of the plot are divided by an inflection point, t_{inf} , which was determined as an N_τ value corresponding to intersection point of two regression straight lines. In this case $t_{inf} \approx 2090$ events. The data in Figure 1b strongly suggest that the channel openings and closings are correlated in time, i.e., there is memory in this kinetic process. As expected, the random shuffling of the data set removes the correlation, and H_{tot} became 0.52 ± 0.03 (Figure 1b).

Figure 1. Activity of single fast K_{CA} channel (a) and its analysis by rescaled range method (b, c). In (a) a fragment in the upper trace indicated by the bracket is presented on a spanned time scale in the bottom trace. Upward deflections refer to channel openings. (b) The Hurst plots for an original array of 2^{16} adjacent τ_0 's and τ_c 's before (open circles) and after (filled circles) randomization. Real time intervals – 1000 events = 4.5 s; $t_{inf} = 9.4$ s. (c) The Hurst plots for the data sets of only τ_0 's (circles) and only τ_c 's (squares) each of 2^{15} events. The open and closed events were selected from the original array in (b). Real time intervals – 1000 events = 9 s; $t_{inf} = 8.1$ s for τ_0 and 18.4 s for τ_c . The lines are the best fits to the experimental points. $V_m = +20$ mV, $[Ca^{2+}]_i = 3.3$ μ M. Real time was determined as $((\bar{\tau}_0 + \bar{\tau}_c)N_\tau)$, where $\bar{\tau}_0$ and $\bar{\tau}_c$ are the averaged values of τ_0 and τ_c . Low pass filter 2.0 kHz.

The analysis of the reduced time series (the sequences of only open or only closed times) gives similar results (Figure 1c). For the data set consisting of only τ_0 's $H_1 = 0.66 \pm 0.02$, $H_2 = 1.27 \pm 0.04$, and $t_{inf} \cong 900$ events; for the series of closed times $H_1 = 0.58 \pm 0.01$, $H_2 = 1.04 \pm 0.04$ and $t_{inf} = 2048$ events. The data show that in this case the open events as a whole are correlated more strongly than the shut events.

Using 69 trial data sets obtained from 10 membrane patches averaged values of H_1 and H_2 were obtained. For the data series consisting of adjacent open and closed events ($\{\tau_0, \tau_c\}$) the averaged Hurst coefficients were as follows: $\bar{H}_1 = 0.60 \pm 0.04$ ($n = 69$), and $\bar{H}_2 = 0.88 \pm 0.21$ ($n = 41$). For the data series consisting of only τ_0 's $\bar{H}_1 = 0.63 \pm 0.10$ ($n = 69$), and $\bar{H}_2 = 0.93 \pm 0.23$ ($n = 61$). The data sets composed of only τ_c 's were characterized by $\bar{H}_1 = 0.60 \pm 0.02$ ($n = 69$), and $\bar{H}_2 = 0.91 \pm 0.22$ ($n = 52$). Approximately one third of the data sets $\{\tau_0, \tau_c\}$ had H_2 lesser than 0.5 (Figure 2), with averaged $H_2 = 0.37 \pm 0.15$ ($n = 25$), that

Figure 2. An example of the $(R/S - N_\tau)$ - dependence for $\{\tau_c\}$ with $H_2 < 0.5$. H_1 and H_2 are indicated in the figure. $V_m = 10$ mV, $[Ca^{2+}]_i = 10$ μ M. 10^3 events $\cong 5.8$ s of real time. $t_{inf} = 4000$ events (~ 23.2 s).

is, for these cases the channel activity at long lags could be characterized as an antipersistent process ([11, 15, 20]). For the reduced data sets consisting of only τ_0 's or τ_c 's the second Hurst exponent $H_2 < 0.5$ occurred more rarely and was 0.27 ± 0.12 ($n = 6$), and 0.31 ± 0.15 ($n = 16$), respectively. $H_2 < 0.5$ (or $H_2 < H_1$) was not an overall characteristic of a given channel under experiment. It could randomly arise both at the beginning and in the middle or at the end of the channel activity. There was no rigorous correlation between H_2 and any channel characteristics (τ_0 , τ_c , etc.). However, in 80% cases $H_2 < 0.5$ appeared when the channel activity was comparatively high ($P_0 = 0.5 \pm 0.18$ vs. $P_0 \cong 0.2$ in other experiments).

H_1 found by us is somewhat lower than overall H found by Hurst [11] for different natural phenomena (0.73), and by Nogueira et al. [8] for the Ca^{2+} -activated K^+ channel in Leyding cells (0.75), or H_1 determined by Churilla et al. [15] for the membrane potential fluctuations in human T -lymphocytes (0.76). On the other hand, H_2 derived from our experiments (~ 0.9) is greater than the Hurst coefficient in the above mentioned works.

The availability of two different slopes of the R/S on N_τ dependencies is somewhat unusual Hurst himself [11] had deal only with the processes being described by one straight line. Bassingthwaite and Raymond [12] in their evaluation of the R/S -methods showed that the fractal Brownian noise is described by the Hurst dependence with a single slope. The same result was obtained by Nogueira et al. [8] in their investigations on K_{Ca} channel in Leyding cells. On the other hand, a pos-

Figure 3. Histogram of inflection points, t_{inf} , for 69 trial data sets from 10 membrane patches. N_τ values corresponding to the inflection points are expressed in real time of the K_{Ca} channel activity.

sibility of inflection of the Hurst plot was allowed by Feder [20], and two slopes of the R/S on N_τ dependence for the membrane potential fluctuations were observed by Churilla et al. [15]: at short lags the Hurst exponent was 0.76, and at long those it was about 0.26 (the latter is close to $H_2 = 0.27 \div 0.37$ found by us in limited cases). Generally, two slopes in the Hurst plots may be indicative of existence of two, at least, short-range, and long-range regimes of the event correlation.

Thus, the data presented show that activity of the K_{Ca} channel in the *Vero* cells as a whole is least three regimes of the correlations. At comparatively short ranges the original sequences of single channel events have moderate positive correlation ($\bar{H}_1 = 0.6 \div 0.67$), and at long ranges the events are strongly correlated either with positive ($\bar{H}_2 = 0.88 \div 0.93$) or negative ($\bar{H}_2 = 0.27 \div 0.37$) correlation.

The characteristic length (t_{inf}) of the data sets corresponding to a transition from a slightly correlated mode to strongly correlated one have polymodal shape (Figure 3). It seems likely that there are several discrete characteristic lengths of the sets corresponding to about 1.0, 2.0, 6.0, 18.0, and 60.0 s. As a whole, the time intervals of correlated channel activity extend from seconds to hundreds of seconds. In all cases the degree of the correlation is high ($\bar{H}_2 \cong 0.9 \pm 0.2$). However, the number of the experiments with $t_{inf} \cong 18$ s is the greatest. An analysis showed that there is some correlation between t_{inf} and P_0 values. Due to a great data scatter, it is difficult to determine distinct dependence of t_{inf} on P_0 . However, a tendency is clearly observed: the more P_0 , the lesser t_{inf} . For example, in one of the experiments an increase of P_0 from 0.001 to 0.11 (due to shifting of V_m from -10 to $+40$ mV at $[Ca^{2+}]_i = 1 \mu M$) was accompanied by a decreases of t_{inf} from

Figure 4. R/S analysis of K_{Ca} channel activity on arrays of different length for alternative τ_0 and τ_c events. (a) An example of dependence of estimated Hurst exponents H_1 and H_2 on the data set length. Increasing arrays of 256 to 10240 events with a step of 256 events were used. Open and closed circles numbered as 1, 2 and 2', 3 and 3' correspond to the data set lengths of 1792 (1), 2048 (2), and 5632 (3) events, for which the Hurst plots are presented in (b–c) and numbered by 1 to 3. $[Ca^{2+}]_i = 10 \mu M$, $V_m = +20$ mV, averaged values of τ_0 and τ_c equal 6.4 ms and 3.5 ms, respectively. Real time intervals: 1000 events $\cong 4.5$ s; t_{inf} equals 1.2 s in c and 3.5 s in d.

570 to 10.5 s. In other case, at $V_m = 0$ mV, when P_0 was increased from 0.012 to 0.8 due to an increase in $[Ca^{2+}]_i$ (1 to 33 μM), t_{inf} decreased from 60 to 0.9 s. The data in Figure 3 were obtained at a comparatively low averaged P_0 , 0.2 ± 0.2 , that corresponds to the most probable t_{inf} value, ~ 18 s. If the experiments were carried out at the P_0 values close to 1, the most probable t_{inf} value could be shifted in the range of seconds. Any physical mechanism of the phenomenon is yet unknown, however, the correlation in the channel activity observed at different discrete time scales resembles fractal processes.

Dependence of H on sample length. Evaluation of accuracy of the rescaled range analysis have shown that in the case of fractional Brownian signals the estimated H values monotonically approach to its true value with sample length [12]. For example, for a true Hurst exponent equals 0.5, the estimated H values are 0.442 ± 0.07 and 0.52 ± 0.04 obtained on arrays of 256 and 4096 points, respectively [12]. For this reason studying the H dependence of τ for the K_{Ca} channels was made. Figure 4a demonstrates the data obtained on the trials with increasing length. The first point of the plot corresponds to the data subset of 256 events. Each successive data subset was formed from a general data set by addition of the next 256 events to preceding one. As is seen, for data lengths of 256 up to 1792 events the estimated H values lies in the vicinity of 0.6 that is close to $H = 0.5$ for a random process. In these cases the dependencies R/S vs. N_τ were approximated rather well by a single straight line with a slope of 0.56 to 0.59 (point 1 in Figure 4a, Figure 4b). However, an addition of further 256 events to previous 1792 events sharply changes the picture: a second, more high slope (0.85) in the plot R/S vs. N_τ appears (Figure 4c), whereas H_1 (0.54) is close to 0.5 as before (points 2 – 2' in Figure 4a). At further increasing the data set length the Hurst plots retain two slopes, H_1 remaining close to 0.6, and H_2 varying in the vicinity of 1.2 (for example, points 3 – 3' and Figure 4d). Appearance of H_2 in Figure 4 is not, more likely, accounted for improvement of the statistics due to the data set elongation, as was observed on the fractional Brownian noise because estimated H values approach the true ones even at the data set lengths of about 200–300 [12]. On the other hand, high value of H_2 observed in Figure 4a is far apart maximally allowable true H value, 1. It may be due to large errors in determination of H when true H is close to zero or 1 [12]. It is of interest that the trial of 256 events by itself lying between 1792 and 2048 events and drastically changing the dependence of R/S on N_τ (the points in Figure 4a denoted as 1 and 2) is characterized by a single slope $H_1 = 0.51 \pm 0.05$ corresponding to a random sequence. Great diversity of the dependencies similar to that in Figure 4a were observed in the experiments.

Thus the results obtained showed that the events of single channel activity are slightly correlated on short time series ($H_1 = 0.5 \div 0.7$), and greatly correlated on large time series (H_2 achieves about 1). The transition zone between the two regimes of the channel activity may be narrow (Figure 4). It is possible that the characteristic times, divided these two zones, are described by the t_{inf} distribution presented in Figure 3.

Simulation of single channel data. Properties of the K_{Ca} channels in *Vero* cells (conductance, sensitivity to Ca^{2+} , kinetic parameters etc.) resemble to some extent those in rat skeletal muscle (cf. [16] and [18]). McManus and Magleby [18] developed the simplest eight-state Markovian model (see Methods) that could describe the major features of the Ca^{2+} -dependence of P_0 , the dwell-time distributions, and the relationship between the duration of the adjacent intervals. We used this model to simulate the single channel data for their Hurst analysis by the R/S method (two state and three-state Markovian channel models were tested earlier

Figure 5. R/S analysis of simulated single-channel data. Upper trace (a) shows the results of simulation of eight-state model (see Materials and Methods) with the same rate constants as those determined by McManus and Magleby for the rat muscle K_{Ca} channel at +30 mV (Table I, channel 1 [18]). Upper deflection refer to channel openings. For the model, $[Ca^{2+}]_i = 29 \mu M$, $P_0 = 0.63$. The simulated channel activity shows bursting character. (b) Calcium dependencies of P_0 for the model channel (filled circles) and for one of the *Vero* K_{Ca} channels. The data for the model channel were obtained by averaging over 10 trials for each plot point. The data scatter is very negligible. For the model channel an apparent dissociation constant ($K_{0.5}$) is $22.03 \mu M$, and the Hill coefficient is 2.64; for the *Vero* K_{Ca} channel $K_{0.5} = 34.5 \mu M$, and $n = 2.68$. $V_m = +20$ mV. The values of $K_{0.5}$ and n were determined by fitting the Hill equation [18] to the data with the least square minimization. (c) Double-logarithmic plots of R/S vs. N_τ for the simulated data (filled circles) and, as a compare, the similar dependence for the τ_0 's of the *Vero* K_{Ca} channel. For the model, $H = 0.52 \pm 0.02$, for the *Vero* channel, $H_1 = 0.66 \pm 0.02$, $H_2 = 1.13 \pm 0.03$; $V_m = +20$ mV, $[Ca^{2+}]_i = 10 \mu M$. Real time intervals: 1000 events = 9.9 s; $t_{inf} = 3.3$ s.

by Nogueira et al. [8]). The results obtained are as follows (Figure 5). Firstly, we assumed that properties of the simulated data correspond to those embedded into the model. Thus, we found that the simulated data gave $K_{0.5} = 22.0 \mu M$, and $n = 2.64$ that is very close to $K_{0.5} = 20.9$ and $n = 3.1$ for the muscle K_{Ca} channel at +30 mV (Table I, Channel 1 in [18]) whose rate constants were used in the model. Further, in this relation the model channel was close to the *Vero* K_{Ca} channels ($K_{0.5}$ was, in average, $27.0 \mu M$, and $n \approx 3.5$ at +30 mV), as is seen in Figure 5b.

One thousand simulations each of 10^4 events were made and value of the Hurst exponent was calculated for each of these simulations on the base of the linear regression in the double-logarithmic plots of R/S vs. N_τ . The straight line is the best fit for the simulated data (Figure 5c). For comparison, the R/S on N_τ dependence

for the *Vero* K_{Ca} channel is presented in Figure 5c. The distribution of the model H values was Gaussian with the mean 0.542 ± 0.023 (S.D., $n = 1000$). This result indicates that, as expected theoretically for the Markovian models, the process is random, memoryless. On the other hand, bursting activity, in a manner as it is formed by the model channel (Figure 5a), could not be responsible for $H > 0.5$.

Recordings of actual channel activity are always subjected to low frequency filtration (filtering with a low pass filter), and such filtering could produce false correlation in time signals [12]. To estimate possible contribution of the filtration to H value, we determined the Hurst exponents for the prefiltered model signals. It was found no effects of the filtration up to, at least, 100 Hz.

The data presented show that single K_{Ca} channel gating, as a whole, is a correlated, persistent process, with slight correlation at short lags ($H_1 = 0.6$) and strong correlation at long lags ($H_2 = 0.88$). In other words, the channel activity is a process with memory, when the state of the channel at each instant depends on the state at the previous instant. Such an apparently random process may be characterized as deterministic chaos.

The data are, probably, not in severe contradiction with the Markovian assumption on the channel kinetics [1] derived from conditional distributions of adjacent open and closed intervals because in our experiments at short lags the process was almost random.

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References

1. McManus, O.B. and Magleby, L.K.: Kinetic time constants independent of previous single-channel activity suggest Markov gating for a large conductance Ca^{2+} -activated K^+ channel, *J. Gen. Physiol.* **64** (1989), 1037–1070.
2. Rothberg, B.S., Bello, R.A. and Magleby, K.L.: Two-dimensional components and hidden dependencies provide insight into ion channel gating mechanisms, *Biophys. J.* **72** (1997), 2524–2544.
3. Liebovitch, L.S. and Toth, I.: A model ion channel kinetics using deterministic chaotic rather than stochastic processes, *J. Theor. Biol.* **148** (1991), 243–267.
4. McManus, O.B., Blatz, A.L. and Magleby, K.L.: Inverse relationship of the durations of adjacent open and shut intervals for Cl and K channels, *Nature* **317** (1985), 625–627.
5. McManus, O.B., Weiss, D.S., Spivak, C.E., Blatz, A.L. and Magleby, K.L.: Fractal models are inadequate for the kinetics of four different ion channels, *Biophys. J.* **54** (1988), 859–870.
6. Liebovitch, L.S. and Sullivan, J.M.: Fractal analysis of a voltage-dependent potassium channel from cultured mouse hippocampal neurons, *Biophys. J.* **52** (1987), 979–988.

7. Geletyuk, V.I., Kazachenko, V.N. and Tseeb, V.E.: Quantized kinetic parameters of single ion channels, *Dokl. Acad. Nauk USSR* **301** (1988), 465–469.
8. Nogueira, R.A., Varanda, W.A. and Liebovitch, L.S.: Hurst analysis in the study of ion channel kinetics, *Brasilian J. Med. Biol. Res.* **28** (1995), 491–496.
9. Liebovitch, L.S. and Toth, T.I.: Fractal activity in cell membrane ion channels, *Ann. N.Y. Acad. Sci.* **591** (1990a), 375–391.
10. Liebovitch, L.S. and Toth, T.I.: Using fractals to understand the opening and closing of ion channels, *Ann. Biomed. Engr.* **18** (1990b), 177–194.
11. Hurst, H.E.: Long-term storage-capacity of reservoirs, *Trans. Am. Soc. Civ. Engrs.* **116** (1951), 770–808.
12. Bassingthwaighe, J.B. and Raymond, G.M.: Evaluating rescaled range analysis for time series, *Ann. Biomed. Eng.* **22** (1994), 432–444.
13. Feller, W.: The asymptotic distribution of the range of sums of independent random variables, *Ann. Math. Statistics* **22** (1951), 427–432.
14. Teich, M.C., Heneghan, C., Lowen, S.B., Osaki, T. and Kaplan, E.: Fractal character of the neural spike train in the visual system of the cat, *J. Opt. Soc. Am. A* **14** (1997), 3529–3546.
15. Churilla, A.M., Gottschalke, W.A., Liebovitch, L.S., Selector, L.Y., Todorov, A.T. and Yeandle, S.: Membrane potential fluctuations of human T-lymphocytes have fractal characteristics of fractional Brownian motion, *Ann. Biome. Eng.* **24** (1996), 99–108.
16. Kazachenko, V.N., Geletyuk, V.I., Chemeris, N.K. and Fesenko, E.E.: Single Ca^{2+} -activated K^{+} channels in cultured kidney cells *Vero*, *Biophysics* **41** (1996), 1359–1370.
17. McManus, O.B. and Magleby, K.L.: Kinetic states and modes of single large-conductance calcium-activated potassium channels in cultured rat skeletal muscle, *J. Physiol.* **402** (1988), 79–120.
18. McManus, O.B. and Magleby, K.L.: Accounting for the Ca^{2+} -dependent kinetics of single large-conductance Ca^{2+} -activated K^{+} channels in rat skeletal muscle, *J. Physiol.* **443** (1991), 739–777.
19. Clay, J.R. and DeFelice, L.J.: The relationship between membrane excitability and single channel open-close kinetics, *Biophys. J.* **42** (1983), 151–157.
20. Feder, J.: *Fractals*, Plenum Press, N.Y., 1988.